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Marking Technique for Larvae

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Abstract.—A technique for marking insect larvae as individuals is described and the kinds of data derivable from studies of individually coded larvae are discussed. The relative importance of larval stages is discussed.

INTRODUCTION

Biological explorations of plants and of insects still suffer from defects of omission. In the case of plants, the roots have been too often ignored (Cody 1986). In the case of insects, the immature stages have in general been much less studied than the mature stage (adult). This understandable but unfortunate bias in scientific effort limits the ultimate value of many population dynamic studies. If key factors controlling population dynamics act during the life stages not studied, then analyses of dynamic data remain either inconclusive or misleading. Hypotheses depending on population dynamic data, such as those involving population genetics, suffer accordingly. Control programs designed without accurate knowledge of the factors affecting immature stages may have minimal effects on target population sizes.

Not only do population numbers often depend on phenomena occurring during the immature stages, but the larval stage is frequently where the physical size achieved by an adult insect is determined. For holometabolous insects that are short lived as adults (the vast majority of Lepidoptera, for example), the mature larval size (at least of females) relates directly to reproductive capacity and therefore to potential for population increase. The last larval stadium is where most of an insect's weight is gained. In the case of *Euphydryas editha* (Boisduval) (Lepidoptera: Nymphalidae), for instance, 60 to 80% of the mature larva's weight is gained then (Weiss, White, & Murphy unpublished). Therefore study of this single stadium may contribute disproportionately more to knowledge of the biology of this and similar insects than would the study of other stadia or stages.

For field study of phenomena such as mortality, growth, and dispersal it is necessary to reliably identify individuals. For adult Lepidoptera this is commonly done by means of some variation of the magic marker technique (Ehrlich & Davidson 1960, Brussard 1970, Scott 1975, Singer & Wedlake 1981, Gall 1985). A number of workers have used techniques for marking larvae, but such studies remain the exception and techniques such as multilation are still current (Weseloh

1985). Here we describe a technique for marking individuals that works within a stadium for larvae of insects.

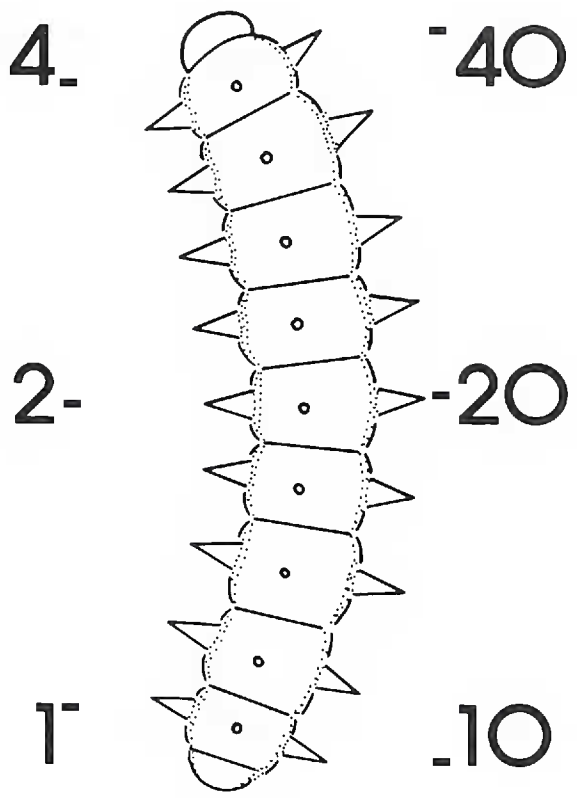
Description of Technique

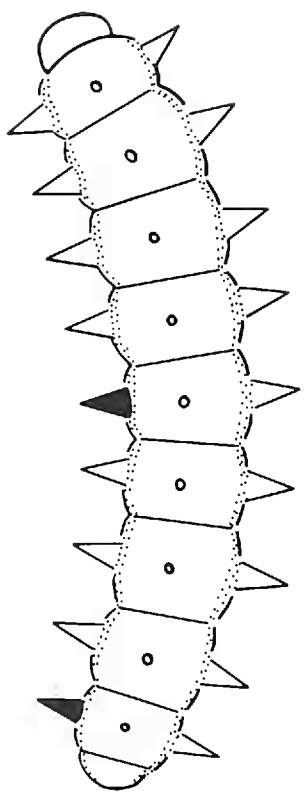
Larvae of *Euphydryas editha bayensis* Sternitzky were collected at the Morgan Hill (MH) site in Santa Clara County, CA, USA, in February and March 1985 and January through March of 1986. Each larva was given a unique mark with Testors enamel paint (available through such outlets as Long's Drugs and hobby shops). Among the dozens of colors available, several worked well with these dark-colored larvae: 1103 red, 1108 light blue, 1114 yellow, 1145 white, 1127 orange, and 1134 purple. On these larvae, 1111 dark blue and 1124 green were not easily readable. Usable light blues and greens could be produced by mixing the darker colors with white. For lighter colored larvae, colors such as dark blue, green and black might be effective. Metallic colors such as silver and gold cause violent reactions: vomiting and fleeing. Testors 1170 light tan was too runny, producing large, messy marks.

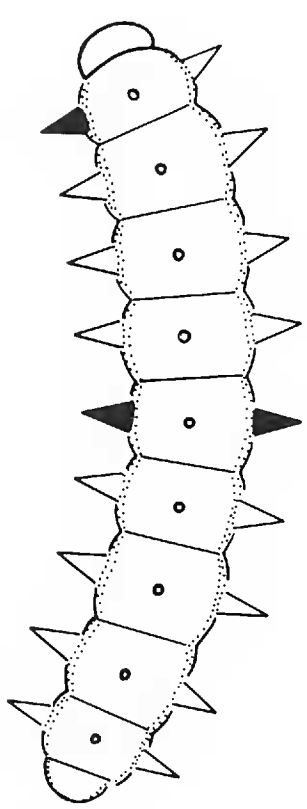
Though there are thirteen body segments in the larvae of Lepidoptera that can, with care, be identified (Howe 1975), we found that we could reliably distinguish dots of paint on the left and right sides of segments A) near the head, B) near the middle, and C) near the end. Very small dots of paint were applied with a sharpened toothpick or with an insect pin (#3) to the subdorsal and/or lateral scoli (bristles) of the appropriate segments. The paint did not wear off, nor did it seem to affect behavior. Marked larvae were picked up 2 to 7 days after marking and release. So far the maximum observed duration of the marks in the field is 21 days, on 2 larvae that had hung up to pupate. Larvae that died of unknown causes and larvae that were stepped on were found to retain their marks identifiably. In this species the final stadium lasts just long enough (7 to 14 days during sunny weather) to be studied. Keeping records of individual morphological traits where they exist, along with painted codes, can help maintain the integrity of the system. Codes, by their nature, are inevitably misread at some frequency.

When the last larval stadium ends and the skin is shed, it usually remains with the pupa. Careful examination of shed skin allows some codes to be distinguished from others (different colors, anal vs. cephalad, left vs. right; potentially also ventral vs. dorsal). Thus individual pupae can be matched to larvae whose traits have been measured.

By assigning numbers (Fig. 1) to the marking positions, each larva could be coded with any of fifty different numbers, without changing colors of paint. It is, however, better for the organism, the experiment, and the investigator to impose as few spots of foreign material as possible. Restriction of the marking scheme to a single dot of paint would allow six unique marks per color of paint used. Restriction to two dots would allow 21 unique marks per color, three dots would allow 40 marks per color, four dots would allow 50. Thus, a three dot system, given six positions and six colors, provides for unique codes to be given to 240 larvae. Obviously the system can be extended by careful use of additional marking locations (eight positions would provide for 444 unique marks). Two colors can be used together on the same individual to, in effect, add another color to the system.







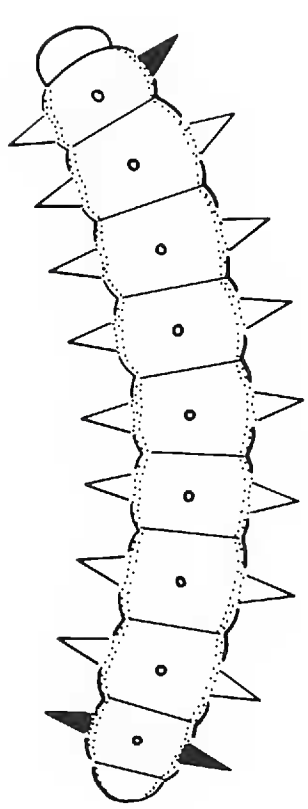


Figure 1. System for coding insect larvae individually. Upper left, key; others, examples.

Uses for Marked Larvae

Marking larvae individually makes it possible to track weight gains of individuals through time under field or laboratory conditions. This is very important because other sampling methods are subject to significant error. For example, we (Weiss, White, & Murphy, unpubl.) have taken field samples of larvae every ten days to assess field growth rates. The resulting data generally show steady growth, but exceptions occur. Even the steady growth curves misrepresent growth to some degree because males lose weight in preparation for pupation while females are still gaining. Preliminary data indicate that in this species the mature larva at its maximum will lose 20% of its weight by the time the pupa is formed (larva, 400 mg: pupa, 320mg). Since males and females of most holometabolous insects differ significantly in size, the problem is a common one. Larvae may be kept individually without marking in the laboratory, but laboratory studies of heliothermic insects are often negatively influenced by the effects of replacing solar heat with ambient heat. Migration of larvae, perhaps surprisingly, can also bias field samples of anonymous (previously unidentified) larvae. Checkerspot butterfly larvae may commonly move ten meters per day when experiencing adverse local conditions. For other experiments, groups of marked larvae can be kept together in the laboratory without the loss of individual identification. The behavior of particular individuals can be tracked over time. Individuals may differ also in other ways (color morphs, for instance) that may affect their growth or survival. Such traits may be recorded if individuals are uniquely identified.

Finding Larvae

When larval hosts of phytophagous insects are known they can be searched at the right time of year and larvae can often be found. However, for many species this works poorly at best, and for many other species it does not work at all or the host is still unknown. In the case of *Euphydryas editha bayensis* (the bay checkerspot butterfly), the host is so common and dense that searching the host plants is very similar to searching the entire habitat. These dark larvae can be found by looking into one's shadow. The spots that remain dark when in shadow may be larvae. These animals are essentially thermal collectors and spend a lot of time basking in areas of the sparsest vegetative cover. It is thought that speed of digestion is a limiting factor, requiring time spent in the sun (Porter 1982). In the case of *E. editha rubicunda* (the large collinsia checkerspot), the postdiapause larvae seem to be crepuscular, feeding at dusk and dawn (Singer, pers. comm.). My own recent experience with isopods, earwigs, and weevils on my garden *Passiflora* reminds me to emphasize the potential value of night searches. The serious investigator may have to be active at midnight with a powerful flashlight. He may also have to lie prone on a muddy substrate (as one of us did this January) in order to see larvae that weigh under 20mg, but that have already doubled in weight since breaking diapause.

Summary

All too often the more difficult life stages of experimental organisms are ignored in terms of literature discussion as well as experiment. Marking individuals of such stages is an invaluable tool for extending knowledge of the biology of insects. The

technique described here is usable on the later instars of many species of insects. So far we have found differential dispersal of larvae on slopes of different exposure and have determined growth rates under some field conditions (Weiss, White, & Murphy, unpubl.). We expect to use the technique described here for further growth, microhabitat, and dispersal studies and to estimate larval survival to pupation.

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